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Genetic Control of Prophage Induction in *Haemophilus influenzae* After Exposure to Psoralen Plus Near-UV Light

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Prophage S2 could be induced by psoralen plus near-UV light (PNUV) from a wild-type strain of *Haemophilus influenzae,* from UV light-sensitive strains *uvr-1* and *uvr-2* and PNUV-sensitive strains PSO1 and PSO7, but not from a recombination-deficient strain, *rec-1.* The levels of prophage induction were comparable in the wild type and an ATP-dependent DNase-deficient strain, KW31, even though the PNUV-induced degradation in the latter strain was considerably lower. Prophage induction could be observed even with chloramphenicol present before, during, and 30 min after PNUV treatment.

In *Escherichia coli,* damage to DNA by UV light has been postulated to induce a metabolically diverse set of functions (the so-called SOS functions), all of which are presumed to enhance the survival of the cell or its phages or both (12). The principal evidence for coordinate regulation of SOS functions is their dependence on the *recA*$^+$ and *lexA*$^+$ genotypes (5, 10). That at least some of the SOS functions are induced has been shown by the requirement for new protein synthesis immediately after the inducing treatment (4, 12, 15, 16, 19).

Of the SOS functions in *Haemophilus influenzae,* a low level of Weigle reactivation (M. S. Bamji, M.Sc. thesis, University of Bombay, Bombay, India, 1978) and prophage induction (6) have been observed after UV light exposure. However, *H. influenzae* is not mutable by UV light (8), nor is the UV light-reactivated phage accompanied by any significant degree of mutagenicity (Bamji, M.Sc. thesis). Thus, it appears that there are some differences between the *H. influenzae* and the *E. coli* systems.

Moreover, in *H. influenzae,* DNA repair and prophage induction have been observed after exposure to psoralen plus near-UV light (PNUV), but mutagenesis in the cell or the induced phage could not be detected (M. F. George and N. K. Notani, Indian J. Exp. Biol., in press). In *E. coli,* DNA repair (3) and mutagenesis (7) after PNUV exposure have been observed, and both functions require expression of the *recA* gene.

In this paper we have therefore investigated the requirements for prophage induction after PNUV exposure in *H. influenzae.* Important parallels and differences have been observed in the two systems with regard to the genes involved, the requirement for new protein synthesis, and the role of DNA degradation products in effecting prophage induction.

To identify the genes that are required to be expressed for prophage induction in the *H. influenzae* system, we constructed S2 phage lysogens of the various mutant strains (Table 1). The lysogens were exposed to PNUV, incubated in growth medium for 2 h, and scored for PFU. Figure 1 shows the amount of phage produced after exposure of the lysogens of the wild-type and various mutant strains to psoralen and different doses of near-UV radiation. It can be observed that, of the recombination-defective strains examined, only in the *rec-1* strain was there absolutely no induction of the S2 prophage on exposure of the lysogens to PNUV. This indicates that the *rec-1* gene product is required in prophage induction by PNUV exposure. The *rec-1* gene expression is also required for prophage induction from lysogens by UV light, mitomycin C, and transforming DNA (13, 14). It may be significant to note that prophage induction was normal in the *rec-2* lysogen. This observation suggests that recombinational events per se may not be involved in prophage induction. *uvr-1* and *uvr-2* are UV light-sensitive strains defective in the excision of pyrimidine dimers (9), and PSO1 and PSO7 are PNUV-sensitive mutants (George and Notani, unpublished data). Figure 1 shows that a prophage was induced in these strains, although the level of phage produced was lower than that in the wild type. From this, we may infer that *uvr-1, uvr-2,* PSO1, and PSO7 gene products play a relatively minor role in prophage induction by PNUV. The lower level of phage observed could be due to the DNA repair deficiency in the mutant strains, which may affect later events by conferring on

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the cell a lowered capacity to repair the damage in the prophage.

Upon exposure of *H. influenzae* cells to PNUV, about 50% of the resident DNA label appeared in the acid-soluble fraction, suggesting substantial degradation of the genome. In strains PSO7 and KW31, however, less than 10% of the resident label was found in the acid-soluble fraction (Table 2). Since only low levels of DNA degradation products are recoverable from these strains, they were used to determine whether DNA degradation products have a role in the induction process. It was observed that in both PSO7 and KW31 the phage was induced, although the level in PSO7 was much lower than

TABLE 1. *Description and sources of H. influenzae strains used in the experiments*

Strain	Phenotype	Reference
Rd	Wild type	1
Rd (S2)	Rd lysogen of S2 phage	2
uvr-1 *uvr-2*	Deficient in excision of pyrimidine dimers	9
rec-1 *rec-2*	Recombination deficient in transformation, transfection, and infection	14
PSO1 PSO7	Sensitive to PNUV	George and Notani (this laboratory)
KW31	ATP-dependent DNase	18
S2	Can lytically grow and lysogenize all of the above strains	13; J. K. Setlow, personal communication

TABLE 2. *Amount of degradation of DNA in strains exposed to PNUV*

Strain	% of initial activity[a]
Rd	52
uvr-1	46
uvr-2	27
rec-1	42
PSO1	40
PSO7	92
KW31	95

[a] Acid-insoluble [^{3}H]thymidine-labeled material remaining in cells after 180-min incubation in growth medium after exposure to PNUV.

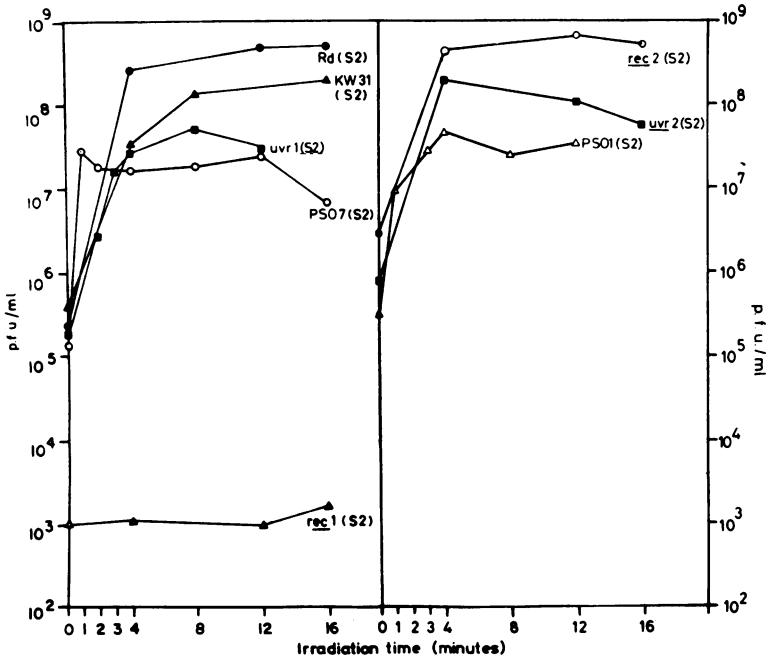

FIG. 1. *Induction of S2 phage in various mutant strains after exposure of the lysogens to PNUV. Exponentially growing lysogenic cells in brain heart infusion broth were suspended in saline buffer at a density of 10^9/ml and exposed to 1 μg of psoralen per ml at 37°C for 15 min and near-UV light (360 nm predominantly) from a Philips HPW 125-W lamp kept at a distance of 25 cm from the sample. The dose rate was 1.2×10^2 J/m^2 per s, and irradiation was done on ice. Samples taken at different times of irradiation were centrifuged, washed, resuspended in brain heart infusion broth at a 1:5 dilution, and grown aerobically at 37°C with aeration. After 120 min of incubation, samples were centrifuged, and the supernatants were assayed for PFU. The 0-min sample was not exposed to psoralen or near-UV light. The figure shows the PFU per milliliter versus time of exposure of lysogens to near-UV-light in the presence of psoralen.*

that in wild-type Rd. The lower induction of phage in PSO7 could have been due to lower amounts of the products of degradation. On the other hand, since in KW31 the level of prophage induction was almost the same as that in the wild type, the lower induction in PSO7 could have been due to the repair deficiency in this strain. This notion is supported by the results observed in mutants PSO1, *uvr-1*, and *uvr-2*, in which the levels of phage induction were lower than that in the wild type, but the degradation was similar to that in the wild type. However, the possibility cannot be discounted that the low levels of DNA degradation products in KW31 are sufficient to induce the prophage.

To determine whether prophage induction in *H. influenzae* is dependent on new protein synthesis, we investigated the effect of protein synthesis inhibition by chloramphenicol. Exponentially growing lysogens were exposed to 50 μg of chloramphenicol per ml for 10 min before, during, and for 30 min after exposure to PNUV. It was observed that, even after a 30-min incubation in chloramphenicol-containing medium, the prophage was still induced, although the levels in PNUV-treated and untreated cells were somewhat lower than those at 0 min of posttreatment incubation with chloramphenicol. These results show that large-scale inhibition of protein synthesis for as long as 30 min after PNUV exposure has no effect on the induction of prophage in an *H. influenzae* lysogen. Although this observation is consistent with the interpretation that S2 phage does not require induced levels of proteins involved in prophage induction, other explanations cannot be ruled out. There is little or no information available regarding the regulation of S2 prophage immunity.

A similar set of puzzling observations was made for *E. coli*. It was found that the presence of chloramphenicol during incubation at 42°C does not prevent lambda phage repressor inactivation in *tif-1* (17) or *dnaB*(Ts) (11) lysogens, nor does chloramphenicol added immediately after gamma radiation prevent repressor inactivation in wild-type lysogens (17), whereas after treatment with UV radiation or mitomycin C, the repressor is not inactivated in the presence of chloramphenicol (15, 16). These observations could indicate that gamma radiation and temperature elevation in temperature-sensitive mutants differ from UV radiation and mitomycin C in the manner of generating an effective SOS signal and that PNUV behaves similarly.

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